Inhibition of Thromboplastin Generation by Hyaluronidase Preparations And Reversal by Lyophilized Platelet Material and Derivatives.* (23781)

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Hyaluronidase has been reported to inhibit blood coagulation in the absence of platelets (1). The depolymerizing effect of hyaluronidase on hyaluronic acid is only slightly reversed by platelets alone, but the combination of platelets and complement or Serum Prothrombin Conversion Accelerator (SPCA) have been shown to inhibit this activity of hyaluronidase as determined by viscosimetric methods(2).

The nature of the inhibitory effects of hyaluronidase on the coagulation mechanism and their relation to platelets, is not known. Studies on the effects of hyaluronidase on the generation of thromboplastin and on the reversal of these effects by platelet material and analogous components from other tissues are therefore reported.

Methods and materials. Bovine testicular hyaluronidase† was suspended in physiological saline or imidazole buffer (pH 7.4). Concentrations were varied from 0.03 to 1.0 mg of hyaluronidase preparation per ml of solution. Fresh platelet concentrates were prepared by differential centrifugation and then washed 6 times in physiological saline. Lyophilized platelet material (LPM) was prepared as described previously(3). Lipid extracts of platelets and of beef brain, containing the thromboplastin-generating activity, were prepared as reported elsewhere(4). Defatted LPM. the residue obtained following extraction of LPM with alcohol-ether (3:1), was used after storage at –20° for periods up to 10 months. The thromboplastin generation test of Biggs and Douglas(5) was modified as indicated in the results.

Results. A thromboplastin-generating mixture produces small amounts of thromboplastin even if platelets or analogous thromboplastin-generating components are not added. In the course of the generation of thromboplastin under these conditions, the clotting time of the substrate plasma decreases from over 2 minutes at the beginning of the incubation to between 35 and 50 seconds after incubating the mixture for more than 10 minutes (Fig. 1).

When hyaluronidase is added to such a platelet-deficient thromboplastin - generating mixture, the formation of thromboplastin is inhibited (Fig. 1).

The addition of fresh platelets or lyophilized platelet material to the hyaluronidase-containing mixture, reverses the inhibition of thromboplastin formation. (Fig. 1). Lipid extracts of lyophilized platelets or of beef brain have similar effects (Fig. 2).

The addition of defatted LPM (free of thromboplastin-generating activity) to a platelet-free thromboplastin-generating mixture containing hyaluronidase, returns the thromboplastin generation to control levels (Fig. 2).

An incomplete thromboplastin generation

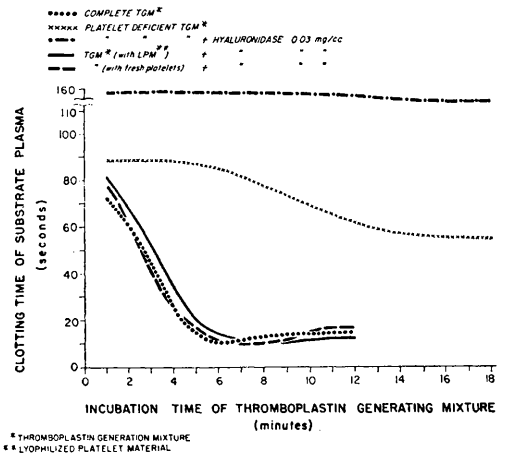

FIG. 1.

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† Worthington Biochemical Sales Co., Freehold, N. J.

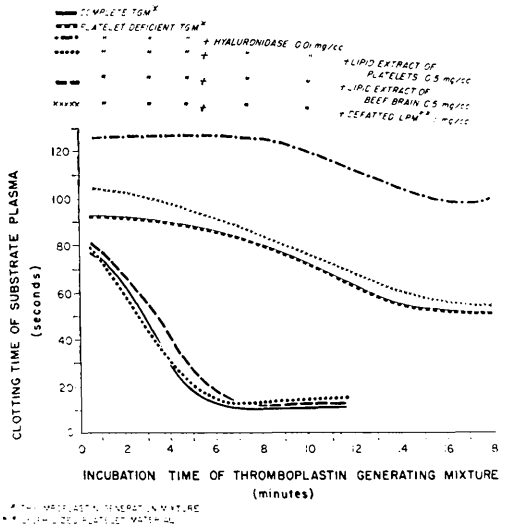

FIG. 2.

mixture, inhibited with hyaluronidase, was incubated for 9 minutes without evidence of thromboplastin formation. LPM was then added and normal thromboplastin formation occurred (Fig. 3). This indicates that hyaluronidase does not destroy an essential component of the coagulation mechanism, but rather acts as a reversible inhibitor.

Discussion. The data presented indicate that bovine hyaluronidase preparations inhibit the generation of plasma thromboplastin in the absence of adequate amounts of platelet material or its equivalent. The generation of thromboplastin after prolonged incubation of a mixture containing $BaSO_4$-treated plasma, serum, calcium ions and buffer (instead of platelets or their equivalent) is probably due to traces of the lipid components of platelets which remain in the serum or the plasma after adsorption with $BaSO_4$ and subsequent high speed centrifugation. Such a system (incomplete thromboplastin-generation mixture) has been found suitable for studies of reactions in which the concentration of one or more platelet or plasma components may be critical.

Since, in our experience, hyaluronic acid and the products of its enzymatic degradation failed to produce significant effects on the generation of thromboplastin, the inhibition reported here may be due to an effect of hyaluronidase unrelated to its depolymerizing activity. This is further suggested by the finding that thromboplastin generation is initiated when platelet material or its equivalent is added to an incomplete thromboplastin-generating system containing hyaluronidase which had been incubated for a period sufficient for the generation of thromboplastin. It should be emphasized that the hyaluronidase preparations used here were relatively impure and the inhibition of thromboplastin generation may have been due to contaminants. On the other hand, the effects described here were common to hyaluronidase preparations from a number of different sources.

In the presence of whole platelets (fresh or lyophilized), as well as of lipid extracts from platelets or brain, the thromboplastin generation reaches the usual levels which are observed in complete thromboplastin generation mixtures. This may be the result of either the direct inactivation of the inhibitory principle in the hyaluronidase preparation, or of compensatory effects of adequate concentrations of thromboplastin-generating agents.

The reversal of the effects of hyaluronidase in the incomplete thromboplastin generation mixture produced by defatted platelet material, free of demonstrable thromboplastin-generating component, suggests a direct mechanism of action. The specific action of hyaluronidase, however, can be inhibited by a large number of agents of biological origin and synthetic compounds(6). A nonspecific interaction of hyaluronidase with some of the nonlipid components of platelets could, therefore, account for the reversal of the effects of

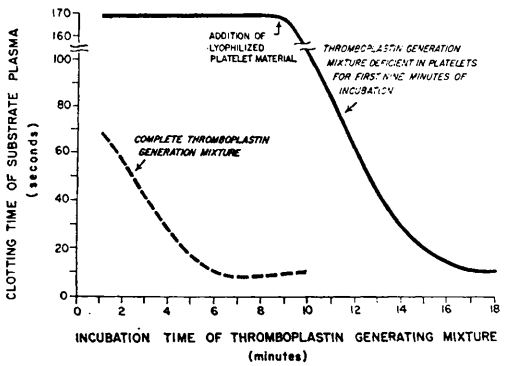

FIG. 3.

hyaluronidase on thromboplastin generation by defatted platelet material.

Although there is no indication that the apparent interactions between hyaluronidase and platelets play any role *in vivo,* further studies in regard to a possible relation of this phenomenon to vascular physiology and pathology may be warranted.

Summary. Bovine testicular hyaluronidase preparations inhibited formation of thromboplastin in absence of platelets or equivalent materials. This effect was not due to destruction of an essential component of the coagulation mechanism. Inhibition was prevented or reversed by platelets, thromboplastin-generating components extracted from platelets and tissues and by defatted platelet material.

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A New Group of Psychotomimetic Agents.* (23782)

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During the past few years, much interest has developed in psychotomimetic agents, particularly with regard to LSD 25 and mescaline. At the same time, considerable emphasis has been placed on the possible role of adrenalin and serotonin in psychoses, particularly because they are structurally related to the psychotomimetic agents and are pharmacologically antagonistic. The role of acetylcholine and acetylcholine-like substances, on the other hand, has received relatively little attention.

Knowledge of the hallucinogenic properties of cholinergic blocking agents, such as atropine and hyoscine, dates back to the time of the ancient Hindus. Recently, a group of piperidyl benzilates possessing anticholinergic properties were synthesized by Biel and associates(1) as possible antispasmodics in the treatment of duodenal ulcer(2). In the course of therapeutic trials, it was found that the tertiary amine hydrochlorides of the benzilate esters, although active anticholinergics, produced undesirable side effects, particularly hallucinations. The quaternary ammonium salts, on the other hand, were entirely devoid of such effects. We have recently obtained a series of such substances and examined their psychotomimetic effects on animals and human subjects(3).

Methods. The psychotogenic effects of the N-methyl-3-piperidyl benzilate and related congeners were tested on over 40 human volunteers who were either normal or patients complaining of minor disorders. Although some of the patients had limited knowledge of the psychotogenic action of the drugs, the majority of subjects were completely unaware of their nature. Ceruloplasmin determinations were made on many subjects, employing a method described previously(4). All of the agents were tested for their behavioral effects in animals, including some 30 Siamese fighting fish, 50 rodents, and 5 cats. The action of these agents on the Siamese fighting fish is comparable to those described for LSD by Abramson(5). In rodents there were marked behavioral changes, such as initial excitement and marked hyperactivity, spontaneous squealing, lack of responsiveness to stimuli, muscular weakness, and lethargy.

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